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<u>L6</u>	65355420.pn. and confocal	0	<u>L6</u>
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L3: Entry 5 of 34

File: USPT

Oct 23, 2001

DOCUMENT-IDENTIFIER: US 6306607 B1

TITLE: Heterogeneous assay for pyrophosphate

Brief Summary Text (22):

In yet another aspect, the present invention provides kits and integrated systems for practicing the assays described herein. In certain aspects, the present invention provides a kit for assaying pyrophosphate cleavage, comprising: (a) a plurality of NTPs each having a .gamma.-phosphate with a distinguishing fluorophore moiety attached thereto and each having a quencher moiety sufficiently proximal to the distinguishing fluorophore moiety to prevent fluorescence of the distinguishing fluorophore moiety; wherein the distinguishing fluorophore moiety exists quenched with at least about a 5 fold quenching efficiency when the .gamma.-phosphate is attached to each of the plurality of dNTP moieties and each is unquenched when the .gamma.-phosphate is detached from each of the plurality of dNTP moieties; and (b) a polymerase. Preferably, the polymerase is immobilized on a solid support.

Detailed Description Text (14):

As such, the present invention provides a heterogeneous assay method for detecting pyrophosphate cleavage, the components of the assay comprising a labeled NTP, a target nucleic acid, a primer nucleic acid and a polymerase, the method comprising: (a) flowing the labeled nucleotide triphosphate (NTP) having a .gamma.-phosphate with a fluorophore moiety attached thereto and a quencher moiety sufficiently proximal to the fluorophore moiety to prevent fluorescence of the fluorophore moiety, past an immobilized component selected from the group consisting of the polymerase and the target nucleic acid; (b) incorporating the NTP on a primer strand hybridized to the target nucleic acid using an enzyme and releasing the .gamma.-phosphate with the fluorophore moiety attached thereto; and (c) detecting the fluorescent moiety thereby detecting pyrophosphate cleavage. In the heterogeneous assay of the present invention, either the polymerase or the target nucleic acid is attached to a solid phase, such as a solid support. Preferably, in the methods of the present invention, the polymerase is immobilized on a solid support.

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L3: Entry 6 of 34

File: USPT

Oct 9, 2001

DOCUMENT-IDENTIFIER: US 6298859 B1

TITLE: Use of a phenol oxidizing enzyme in the treatment of tobacco

Detailed Description Text (98):

In one embodiment, the enzyme used is immobilised and is easily and quantitatively removed, thereby facilitating the product of the invention to be substantially free from the added phenol oxidising enzyme. Common techniques for immobilisation of enzymes are known in the art and include, without limitation, adsorption, covalent bonding and cross-linking onto carrier materials such as ion-exchange resins, artificial polymers, e.g. nylon, polyethylene imine, polystyrene, methacrylate, naturally occurring biopolymers and derivatives thereof, e.g. chitin, chitosan, glyceryl chitosan, cellulose and derivatives thereof, e.g., DEAE-cellulose, (ground/crushed-) egg shells, inorganic materials, e.g. SiO₂, glass beads, bentonite, and other insoluble supports, as well as encapsulation in gels or (micro)capsules prepared from polymers. Typically, immobilised enzymes exhibit little or even no leakage of enzyme during use, resulting in a liquid fraction with no or very little enzyme when separated from the immobilised enzyme particles. A very specific and sensitive method for quantification of the amount of leaked enzyme is the use of immobilized enzyme which has been radioactively labelled prior to immobilisation, e.g. by methylation of lysine residues in the protein backbone by means of reaction with ¹⁴C-formaldehyde. Some examples of immobilisation of enzymes are disclosed in Pialis, P. et al. (1996), Biotechnology and Bioengineering, 51, page 141-147; Bhosale S. H. et al. (1996), Microbiological Reviews, 60(2), page 280-300; Spagna G. et al. (1993), Journal of Chemical Technology and Biotechnology, 57, page 379-385; Spagna G. et al. (1998), Process Biochemistry, 33(1), page 57-62; Martino A. et al. (1996), Process Biochemistry, 31 (3), page 281-285; and Martino A. et al. (1996), Process Biochemistry, 31(3), page 287-293.

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L3: Entry 7 of 34

File: USPT

Jul 3, 2001

DOCUMENT-IDENTIFIER: US 6255083 B1

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TITLE: System and methods for nucleic acid sequencing of single molecules by polymerase synthesis

Detailed Description Text (2):

This invention provides for a method of genotyping or sequencing a target nucleic acid [NA] comprising the steps of: i. immobilizing a nucleic acid polymerase or the target nucleic acid onto a solid support in a single molecule configuration; ii. contacting the solid support with a solution containing: (a) a target nucleic acid where the polymerase is immobilized or a polymerase where the target nucleic acid is immobilized; (b) a primer nucleic acid which complements a region of the target nucleic acid downstream of the region to be sequenced; (c) NTP where each type of base is differently labeled on the phosphate portion, where the labels provide a unique signal that is selectively detectable upon incorporation of NTP into the polymerase extension product; iii. permitting the polymerase to sequentially extend the primer incorporating the NTP creating a complement to the target nucleic acid; and, iv. determining extension of the primer by detecting the unique signal from the labeled NTP to genotype or to sequence the target nucleic acid. The method may use a solution which contains at least two different types of NTP.

Detailed Description Text (3):

It is preferred that the NTPs are labeled on the gamma phosphate with a fluorescent label that differentially fluoresces when the gamma phosphate is cleaved away from the nucleoside. The immobilized moieties can further comprise an array of locations each bearing a single molecule of polymerase or target nucleic acid. The immobilized moiety can be a polymerase positioned as an array of individual, single molecules onto a solid support. For genotyping the target nucleic acid may be a single nucleotide polymorphism. In such circumstances one need only add a solution which has a single type of NTP. For sequencing the detection involves a sequential detection of different types of NTP to provide the sequence of the target nucleic acid. In a preferred embodiment, the dNTP is labeled with a fluorophore on the gamma phosphate and a quencher moiety. In another preferred embodiment the solution contacting the solid support flows past the immobilized polymerase or target NA. The polymerases can be DNA dependent or RNA dependent DNA polymerases or DNA dependent RNA polymerases. The NTPs can be ribonucleotide triphosphates [rNTPs] or deoxynucleotide triphosphates [dNTPs] depending on the target nucleic acid and the polymerase in use.

Detailed Description Text (4):

This invention further includes a system for genotyping or sequencing a target NA comprising: i. a solid support having a surface bearing an immobilized nucleic acid polymerase or the target NA in a single molecule configuration; ii. a solution for contacting the surface containing: (a) a target NA where the polymerase is immobilized or a polymerase where the target NA is immobilized; (b) where the polymerase requires the use of a primer nucleic acid there is added a primer which complements a region of the target NA downstream of the region to be sequenced; (c) a molar excess of NTP, where each type of NTP base is differently labeled on the phosphate portion and where the labels provide a unique signal that is selectively detectable upon incorporation of the NTP into the polymerase extension product. The

system embraces the same embodiments identified above for the methods. Where the system involves flowing solutions, the force for the flow may be mechanically generated or electroosmotically generated using electrodes.